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Phenotypic Expressions of a Gly154Arg Mutation in Type II Collagen in Two Unrelated Patients With Spondyloepimetaphyseal Dysplasia (SEMD)

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Type II collagenopathies consist of chondrodysplasias ranging from lethal to mild in severity. A large number of mutations has been found in the *COL2A1* gene. Glycine substitutions have been the most common types of mutation. Genotype-phenotype correlations in type II collagenopathies have not been established, partly because of insufficient clinical and radiographic description of the patients.

We found a glycine-to-arginine substitution at position 154 in type II collagen in two unrelated isolated propositi with spondyloepimetaphyseal dysplasia and provide a comparative clinical and radiographic analysis from birth to young adulthood for this condition. The clinical phenotype was disproportionate short stature with varus/valgus deformities of the lower limbs requiring corrective osteotomies, and lumbar lordosis. The skeletal radiographs showed an evolution from short tubular bones, delayed epiphyseal development, and mild vertebral involvement to severe metaphyseal dysplasia with dappling irregularities, and hip "dysplasia." The metaphyseal abnormalities disappeared by adulthood.

KEY WORDS: osteochondrodysplasia, spondyloepimetaphyseal dysplasia, collagen, COL2A1, genotype-phenotype

INTRODUCTION

Chondrodysplasias, inherited forms of skeletal diseases, are caused by a diversity of mutations in genes coding for cartilage proteins [reviewed by Horton, 1995]. Based on clinical, radiographic, and morphologic changes, the disorders can be classified as disease families [International classification of osteochondrodysplasias, 1992]. Recent molecular findings confirm the concept of disease families. The type II collagenopathies are due to mutations in various cartilage collagen genes in mice and men, such as *COL2A1, COL9, COL10A1*, and *COL11* [reviewed by Spranger et al., 1994; Ritvaniemi et al., 1995; Prockop and Kivirikko, 1995]. Mutations in fibroblast growth factor receptor 3, *FGFR3*, cause achondroplasia-group disorders; mutations in diastrophic dysplasia sulfate transporter, *DTDST*, cause diastrophic-group disorders; and mutations in cartilage oligomeric matrix protein, *COMP*, cause pseudoachondroplasia-group disorders [reviewed by Horton, 1995]. Since 1989, more than 50 mutations in the *COL2A1* gene coding for a major cartilage protein, type II collagen, have been reported [reviewed by Prockop and Kivirikko, 1995]. The clinical and radiographic phenotypes range from lethal to mild chondrodysplasias [Spranger et al., 1994]. The pedigrees present with either autosomal dominant inheritance or the patients are isolated cases. The types of mutations are also most variable, and, in essence, all families have a mutation of their own. In general, reports on the mutations of *COL2A1* gene include only the principal clinical and radiographic descriptions of the patients, and it has not been possible to establish a consistent genotype-phenotype correlation in mutations of the *COL2A1* gene [Spranger et al., 1994].

We recently reported on a sporadic G-to-A point mutation in the *COL2A1* gene which resulted in Gly154Arg substitution in type II collagen and a spondyloepiphyseal dysplasia (SED) in a 16-year-old boy [Vikkula et al., 1993]. This patient had marked metaphyseal dysplasia as well. Unexpectedly, an identical mutation was found in an unrelated 26-year-old woman with chondrodysplasia. This allowed us to compare

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

the phenotypes recorded on these patients during an extended follow-up from birth to adulthood. The clinical and, in particular, the radiographic phenotypes changed during the evolution of the disease from infancy to adulthood. We observed both similarities and dissimilarities in phenotypes of the two patients. We propose that the condition, spondyloepimetaphyseal dysplasia (SEMD), and other SEMDs from previous reports represent an entity with phenotypic variation among the type II collagenopathies and should not be included in the congenital spondyloepiphyseal dysplasias (SEDC).

STUDY SUBJECTS AND METHODS

Propositi

The propositi were referred as newborn infants to the Helsinki University Children's Hospital, for evaluation of short stature, and were followed from early childhood by the authors (I.K. and E.M.). The methods of clinical and laboratory evaluation and radiographic studies applied were determined by the diagnostic workup and clinical problems of the patients. Most of the pediatric, orthopedic, and clinical genetics services were provided by the Helsinki University Children's Hospital.

PCR Amplification and DGGE Analysis

The EDTA blood samples were collected from patients and their parents with informed consent, and genomic DNA was isolated from cells in 10 ml of blood [Bell et al., 1981]. The genomic DNA was used as a template for PCR amplification of exons 6 to 49 coding for the triple-helical domain of the *COL2A1* gene and the corresponding exon flanking sequences. The PCR products were then screened for mutations by DGGE. The primer sequences, conditions for PCR amplifications, and DGGE analysis have been described previously [Ritvaniemi et al., 1993].

Dideoxynucleotide Sequencing

For nucleotide sequencing of the PCR products containing exon 15 and exon flanking sequences, the fragments were cloned into a pUC18 Sma/Bap vector with a commercial ligation kit (SureClone Ligation Kit; Pharmarcia). The cloned PCR products were analyzed by dideoxynucleotide sequencing (T7 Sequencing Kit; Pharmacia).

RESULTS

Molecular Defect

The DGGE method was used for analyzing the *COL2A1* gene from patient P2. The analysis demonstrated abnormally migrating bands in several PCR products. Most of them were previously shown to be neutral polymorphisms [Ritvaniemi et al., 1993]. Absent from over 200 other alleles from controls and patients with a variety of chondrodysplasias, an additional sequence variation in exon 15 was detected (Fig. 1). However, as indicated in Fig. 1, a similar sequence variation was detected n patient P1. This sequence variation results from G-to-A transition which converts the codon GGG for glycine to a codon AGG for arginine at position α1-154 [Vikkula et al., 1993].

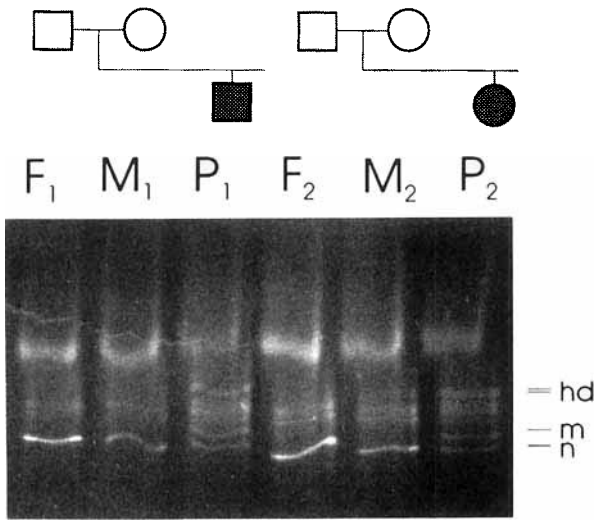


Fig. 1. Analysis by DGGE of exon 15 PCR products from P1, P2 and their parents. The analysis demonstrates a shift in samples from the patients indicating a mutated allele. Similar shifts were not found in over 200 alleles examined from other patients and healthy controls. F = father, M = mother, hd = heteroduplex, m = mutated allele, n = normal allele.

The sequence variation was not detected in the DNA samples from the parents (Fig. 1).

In order to identify the sequence variation in proposita P2, PCR products containing exon 15 and flanking sequences were cloned and sequenced. Sequencing identified a transition that was identical to the one de-

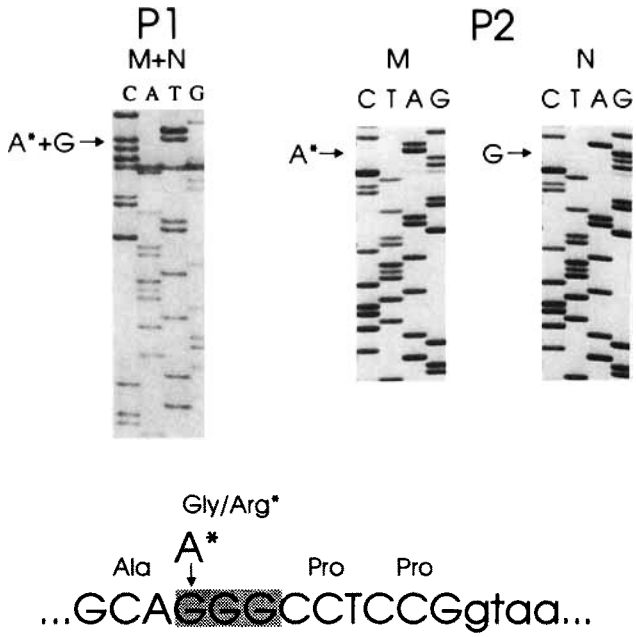


Fig. 2. Direct sequencing of the amplified DNA from P1 demonstrates a G-to-A substitution. NOTE: The sequence shown represents the complementary strand [reproduced with permission from Vikkula et al., 1993]. The sequencing of the mutated and normal alleles of P2 demonstrates the same mutation of glycine (GGG) to arginine (AGG) at position 154 of the triple helix of type II collagen.

tected previously in propositus P1 (Fig. 2). The mutation was demonstrated in 3 independent clones. Thus, as suggested by the DGGE finding, patients P1 and P2 had identical mutations.

The mutation eliminates a Sau961 restriction enzyme cleavage site. The enzyme was used for verifying the mutation in P1 and P2 and for screening the parents for the mutation (Fig. 3). As indicated in Fig. 3, the digestion also confirms that both of these mutations were sporadic, since the parents were homozygous for the normal allele.

Description of the Patients

Propositus P1. Patient P1 (Fig. 4A–D) was described in part previously [Vikkula et al., 1993]. At the time of present clinical evaluation he was a 19-year-old man who had been followed and treated at the Children's Hospital from birth for severe disproportionate short stature and progressive skeletal deformities. The nonconsanguineous parents and his 25-year-old sister and 11-year-old brother were healthy and of normal height, and there was no history of short stature in the family. The mother had had two spontaneous I trimester abortions, and later one induced abortion. P1 was born after an uneventful term pregnancy with a birth length of 49 cm and weight of 4,450 g, but with somewhat short limbs, short neck, and "coarse" face, for which he was referred for evaluation at 2 weeks of age. The skeletal radiographs suggested an unusual skeletal dysplasia with mild platyspondyly, short and thick tubular bones, and pectus excavatum. He had recurrent pneumonia and otitis media in childhood. Early motor development was slightly slow; intellectual development, normal. The growth retardation was associated with progressive varus deformities of the lower limbs with valgus deformities of the ankles, and extreme lumbar lordosis. The shortness of the limbs was rhizomelic, and the hands were normal. At 4½ years he had bilateral corrective distal osteotomy of the femora, and at 5½ years, bilateral corrective osteotomy of the tibiae. To reduce the lumbar lordosis and anteversion of the pelvis he had, at 6 10/12 years, bilateral intertrochanteric osteotomy of the femora; and, due to the recurrence of the lumbar lordosis and unilateral genu valgum, he had corrective osteotomy of the right femur at 12 3/12 years and, at 15½ years, of the left femur. The estimation for expected height, based on parental midheight, was +1.2 SD of the normal mean for Finnish males [Sorva et al., 1984].

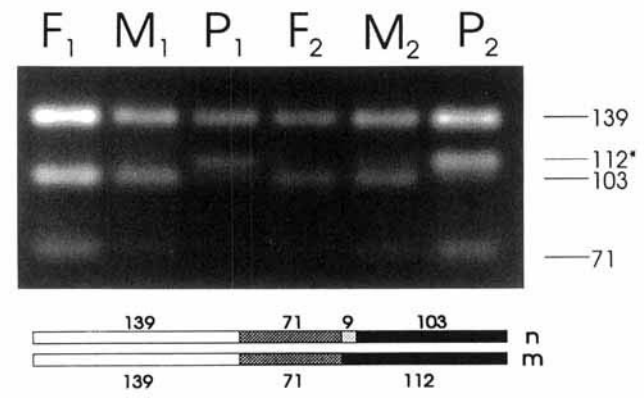


Fig. 3. Sau96I restriction site analysis of exon 15 PCR products from P1, P2, and their parents. The mutation eliminates a Sau 961 restriction enzyme cleavage site. The length of the PCR fragment was 322 bp, and Sau96I digestion normally generates four smaller fragments: 139 bp, 71 bp, 9 bp, and 103 bp. The mutation eliminates the restriction site between the last two fragments (9 bp and 103 bp), generating a fragment of 112 bp. The restriction site analysis revealed similar changes in P1 and P2, but no changes in the parents.

At age 19 8/12 years (Fig. 4C) his height was 130 cm or −7.2 SD of the normal Finnish mean, weight 43 kg or 58% of the relative average weight. Sitting height was 75 cm or 57.7%. There was a mild limp of the right lower limb. The limbs were rhizomelic and only moderately short and disproportionately long to total height. The extension limitations at the right and left elbows were 15° and 20°, respectively; the range of pronation-supination was normal. Hands and fingers were normal. In the lower limbs there were several operation scars, and a marked varus deformity of the right knee which was aggravated by the symmetric laxity of the collateral ligaments of the knees. The range of flexion-extension at the hip joints was normal, whereas inward rotation of the right hip was increased up to 45°, outward rotation limited to 20°, inward rotation of the left hip normal 20°, and outward rotation limited to 20°. There was no pain on passive movement at the hip joints. The feet were normal, but there was a mild valgus deformity in the right ankle. The chest was mildly flattened, and there was a deep asymmetric pectus excavatum. In the spine there was mild lordosis at the thoracal spine, mild thoracolumbar kyphosis, deep lumbar lordosis, but no scoliosis. The head, face, and palate were normal and so were the findings in cardiovascular, respiratory, gastrointestinal, and neurological systems. Secondary sexual characteristics were normal. There was no problem in hearing, and, in ophthalmological consultation, no abnormalities were observed. The patient was studying for the high school maturation examination.

Proposita P2. Patient P2 was the 27-year-old daughter of healthy non-consanguineous parents who had one previous healthy daughter. The parents were of normal height. The father and sister were myopic, −8.5 and −6.5 dp, respectively. The father had one maternal aunt and one maternal uncle with chondrodysplasia and bone fractures at an elderly age. By history their parents had been healthy and of normal height. Based on the physical examination of the aunt at the age of 76 years by one of the authors (I.K.) and on the skeletal radiographs, taken in adulthood, the condition appeared to be a form of autosomal recessive acroosteolysis. The pregnancy history of the mother of P2 documented premature contractures at 4 months of gestation. However, the delivery was induced at 41 weeks of gestation. Birth length was 46 cm and weight 3,820 g, Apgar score 6. Short limbs were noted immediately. She had mild transitory respiratory problems and feeding difficulties for the first 2 weeks. Early motor development was slightly retarded with independent walking at 18

months, whereas intellectual development was normal. Growth was retarded from birth. The first 4 years of life were complicated by recurrent respiratory infections and otitis, and she had an adenotomy. No immunological studies were performed.

By age 5 years she had pain in the lumbar spine, fatigue in walking, and a severe lumbar lordosis. At 6½ years asymmetric genua valga were present and treated at 8 years on the right with corrective tibial osteotomy. Valgus deformity of the right knee re-occurred and resulted in pain, and she had a reosteotomy at 9 years complicated by right peroneus paresis. Problems in the right knee continued, and a reosteomy was required at age 13 years. Only through a fourth tibial

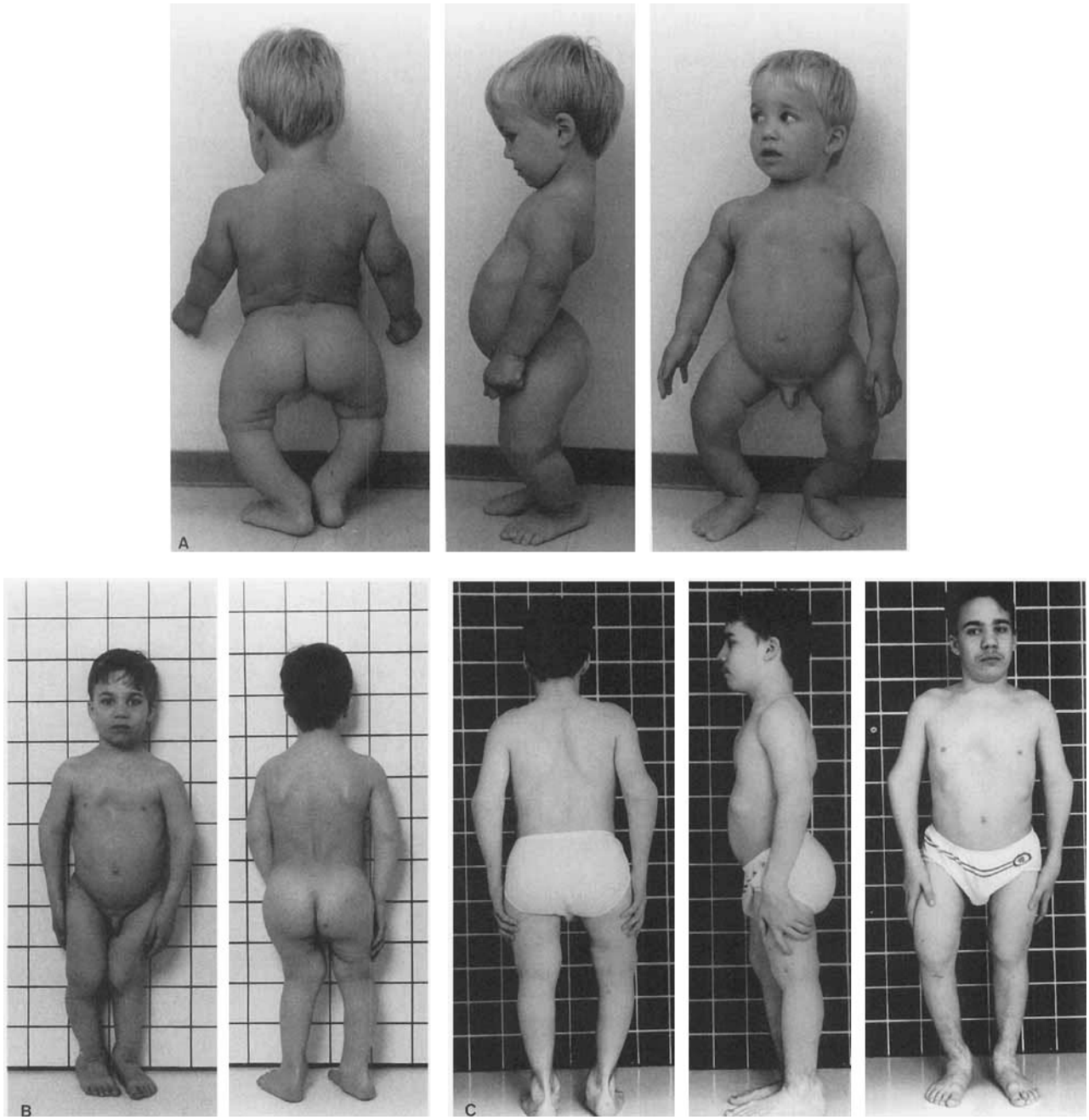
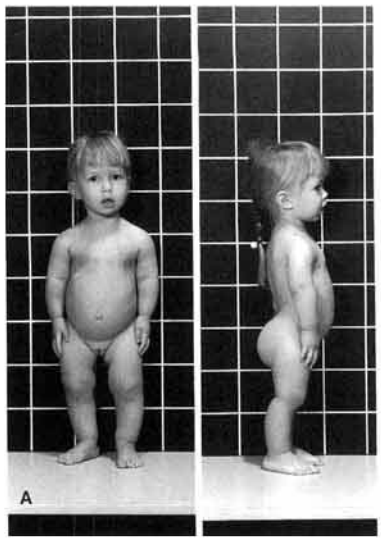
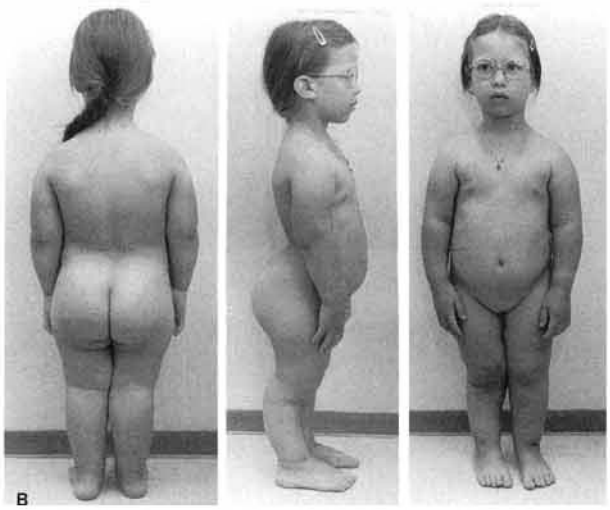
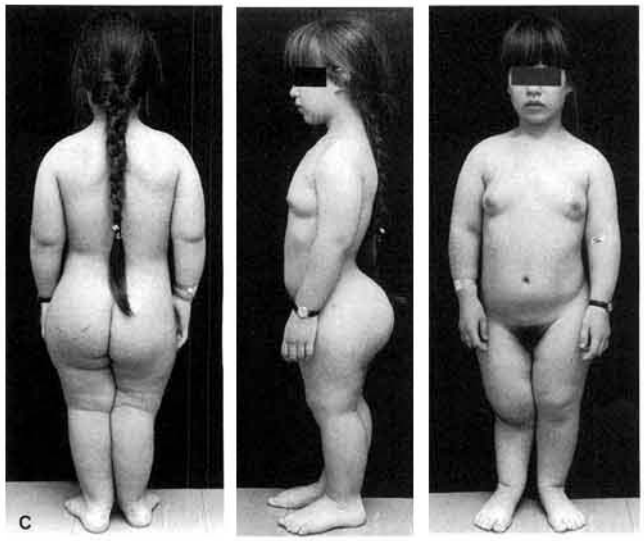


Fig. 4. Patient P1. In a series of clinical photographs with anteroposterior, lateral, and posterior views at age 3¾ years of age **(A)**, at 10 **(B)**, and at 17 **(C)**, he presents with severe disproportionate short stature, increased lumbar lordosis, rhizomelia, extension limitations of the elbows, and normal hands. The severe genua vara have been corrected with bilateral osteotomies of the tibiae and 3 bilateral osteotomies of femora between age 4 and 15 years. Pubertal development is normal. The final height was 130 cm, or −7.2 SD of the normal mean.







osteotomy at age 18½ years a satisfactory result was achieved. Myopia and mild hearing loss were observed at age 9 years. The estimation for expected height, based on parental midheight, was −0.5 SD of the normal mean for Finnish women [Sorva et al., 1984].

At age 27 years the proposita was a university student and tutor in adolescent activities. She had a marked physical handicap because of the severe short-limb short stature, obesity, easy fatigability, and pain the lower limbs. On physical examination she had disproportionately short-limb short stature and moderate obesity. Height was 113 cm, span 117, sitting height 74.2 cm or 65.7%, and weight 51 kg or +150–160% of relative weight. In the spine there was a marked lumbar lordosis. The chest was normal. Hands and feet were proportionately short. There were no limitations in extension or pronation-supination at the elbow joints. The range of movements of the lower limbs was normal except for mild hyperextension at the knees. There was a mild genu varum. Cardiovascular, respiratory, gastrointestinal, and neurological findings were normal except for postoperative peroneus paresis on the right. The ophthalmological consultation documented myopia of −5.5dp O.D. with astigmatism of −4.0 dp, and O.S., myopia of −7.0 dp with astigmatism of −3.0 dp. There was a minimal opacity in the lower aspect of the left lens and two greyish spots in the temporal retina of the right eye, but no indication for retinoschisis or detachment.

Comparison of the Clinical Phenotypes

The evolution of the clinical phenotypes of patients P1 and P2 can be compared in Figures 4A–C and 5A–C. A phenotypic comparison between the condition of the propositi and SEDC and SMED, Studwick type, is also presented in Table I.

The propositi presented with essentially the same severe growth deficit of −7.2 and −7.4 SD of the normal adult mean height. Stature was almost identical with rhizomelic shortness of the limbs, slightly short back, and lumbar lordosis. Both patients had severe deformities of the lower limbs, which resulted in fatigue, pain, and joint limitations. Patient P1 had symmetric genua vara in early childhood (Fig. 4A) which were corrected by osteotomies. In adolescence he had corrective re-osteotomies on the lower limbs. P2 had repeated osteotomies on the right lower limb. The final outcome was not satisfactory for P1, but no procedure is currently scheduled. During the early evolution of the disease both had severe lumbar lordosis which, in part, resolved itself. P1 had a mild thoracic lordosis and

Fig. 5. Patient P2. A series of clinical photographs at age 3 11/12 years **(A)**, 10 years **(B)**, and 13 11/12 years **(C)** demonstrates the disproportionate rhizomelic short stature, moderate obesity, and increased lumbar lordosis. In early childhood she had mild genua vara which later developed to right genu valgum requiring three proximal osteotomies of the right tibia and one osteotomy of the right distal femur between 8 and 19 years of age. Pubertal development was normal with menarche at 11 years of age. Final height was 113 cm, or −7.4 SD of the normal mean.

TABLE I. Phenotypic Comparison of the Propositi (P1, P2), SEDC, and SMED (Strudwick Type)

	P1	P2	SEDC	SMED (Strudwick)
Severe growth failure	+	+	+	+
Chest deformity				
Pectus excavatum	+	−	−	−
Pectus carinatum	−	−	+	+
Spinal deformity				
Scoliosis	−	−	+	+
Kyphosis	+	−	+	+
Lumbar lordosis	+	+	+	+
Odontoid hypoplasia	−	−	+	−
Cleft palate	−	−	+	−
Inguinal hernia	−	−	−	+
Limbs				
Clubfoot	−	−	+	+
Short	+	+	+	+
Genu varum/valgum	+	+	+	+
Normal mentation	+	+	+	+
Cord compression	−	−	+	+
Myopia	−	+	+	+
Retinal detachment	−	−	+	+
Hearing loss	−	+	+	−[a]
Radiographic findings				
Misshaped skull	−	−	+	+
Platyspondyly	+	+	+	+
Metaphyseal flaring and irregularities	+	+	+	+
Metaphyseal corner fracture	+	+	−	−
Dappling of metaphyses	+	+	−	+
Delayed epiphyseal maturation	+	+	+	+
Short tubular bones	+	+	+	+
Normal hands and feet	+	+	+	+
Hip dysplasia	+[b]	+[b]	+	+
Autosomal dominant	−[b]	−[b]	+	+

[a]Not known.
[b]Sporadic.

thoracolumbar kyphosis in adulthood. Neither had any abnormality of the palate. In infancy both had recurrent upper respiratory infections more frequently than their sibs and age-mates, but not to such an extent that basic immunodeficiency would have ever been studied. Retrospectively, the infections had not been caused by opportunistic agents, and the total and differential counts of blood leucocytes had repeatedly been normal.

The differences in clinical phenotypes were normal birth length, normal hand length, pectus deformity, and mild lordokyphotic deformity of the spine of P1, and normal extension at the elbows, myopia and obesity of P2. The normal birth length of P1 might have been due to an error in measurement. Myopia and astigmatism of P2 might be explained by familial liability from paternal side.

Comparison of the Radiographic Phenotypes

The radiographic skeletal evolution is illustrated in Figures 6–11 and the radiographic phenotypes of the patients are compared with those of SEDC and SMED, Strudwick type, in Table I.

The vertebral abnormalities of the propositi are similar with mild platyspondyly, rounding of the vertebral corners, and unmineralized dysplasia of the anterior upper corner of some of the vertebrae. The lumbosacral angle is increased in childhood. The metaphyseal abnormalities are prominent and present with the dap-

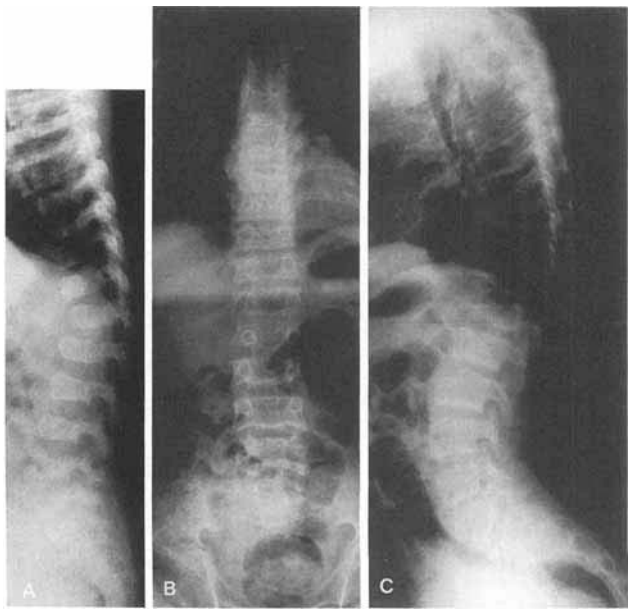


Fig. 6. Spine of P1. In radiographs at age 1 1/12 years **(A)**, 5 years **(B)**, and **(C)** the mild platyspondyly associates, at infancy, with rounded corners; and in adolescence, sclerotic endplates of the vertebral bodies. L1 is hypoplastic and retropositioned, later wedge-shaped. There is a normal caudal increase in the interpedicular distance and L5 spina bifida occulta. In adolescence, the patient has a mild thoracolumbar kyphosis and marked increase in lumbosacral angle.

Fig. 7. Lower limbs and pelvis of P1. The radiographs of the lower limbs at age 2 weeks **(A)**, and at 1 5/12 years **(B)** of the knees at 12 2/12 years **(C)**, and, after corrective osteotomy, at 17 years **(D)**; of the pelvis at 1 5/12 years **(E)**, 10 11/12 years **(F)**, and at 19 years **(G)**. The tubular bones are short and thick. The rounded, broad ends have developed to flared and irregular metaphyses with corner fractures at the proximal medial tibiae and distal lateral femur. At the knees the metaphyses and the epiphyses are irregularly mineralized or dappled. The development of the epiphyses is delayed, and when they appeared at the femoral head, they were small and fragmented. The tibial epiphyses are flattened, and their lateral aspects are fragmented. In adolescence, the left knee is almost normal with mild dappling. The iliac bones are small and rounded, the sacrosciatic notches small, and the acetabular roofs horizontal. The ossification of ilioischial synchondroses and pubic rami is delayed. The lesser trochanters are peculiarly spur-like. The femoral necks develop late and are very short, broad, and in varus position, which results in higher than normal location of the greater trochanters. The femoral heads are severely flattened.

pling and corner fracture phenomena in childhood. They are milder in P1. The development of the epiphyses is markedly delayed, and they are severely dysplastic at the proximal femur. The long tubular bones in the limbs are short and thick, and rhizomelia is clinically and radiographically obvious. The small tubular bones in hands and feet are remarkably normal in spite of slight shortness of the fingers of P2. Skull is normal.

Diagnosis in the Propositi

The clinical diagnostic evaluation of the patients at birth confirmed chondrodysplasia and excluded the most common sporadic types, i.e., achondroplasia, and the two especially common types among the Finns, i.e., diastrophic dysplasia and cartilage-hair hypoplasia. The evaluation and consultations suggested SEDC, which, however, is usually more severe and often includes cleft palate and ocular manifestations. In adolescence the clinical changes were compatible with Kniest dysplasia, SEDC, and SEMD.

Based on mild but distinctive radiographic abnormalities in the spine and epiphyses, and on remarkable abnormalities in the metaphyses, an appropriate diagnosis is SEMD. The dappling of the metaphyses has been considered a diagnostic sign in SMED, Strudwick type [Andersson et al., 1982; Kouseff et al., 1984; Tiller et al., 1993]. The metaphyseal corner fracture phenomenon has been observed in spondylometaphyseal dysplasia (SMD), Sutcliffe type [Langer et al., 1990].

DISCUSSION

Establishing genotype-phenotype correlations in genetic diseases has turned out to be exceedingly compli-

Fig. 8. Upper limb of P1. Radiographs at 2 weeks **(A)** and at 1 7/12 years **(B)** show short and broad tubular bones and rounded ends of the humerus. The metaphyses develop spur-like broadening. The development of the epiphyses is delayed. In the hand at 1 7/12 years of age **(C)** and at 17 years **(D)** the tubular bones are almost normal, whereas the ossification of the wrist bones is delayed, and the bones become dysmorphic.

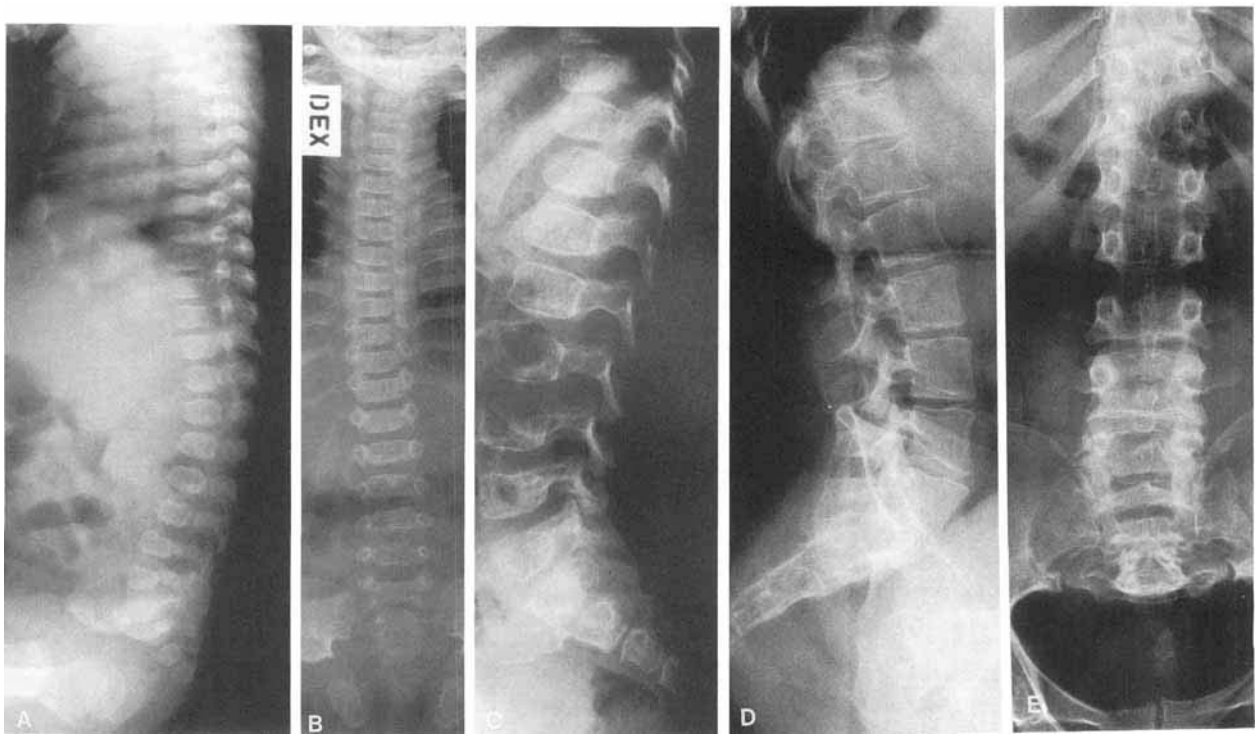


Fig. 9. Vertebrae of P2. Radiographs at 6/12 years **(A, B)**, at 5 3/12 **(C)**, and at 27 years **(D, E)** show marked platyspondyly and, in the lumbar vertebrae, mild anterior beaking. The costochondral junctions are flared, cupped, and sclerotic. From childhood the platyspondyly is less evident, and at 27 years of age the height of the vertebral bodies is almost normal with only mild platyspondyly at the upper lumbar and lower thoracal spine. The upper anterior corner of the vertebral bodies of T10 and T11 is irregular and dysplastic. The lumbosacral angle is markedly increased, and the intervertebral spaces narrow.

Fig. 10. Lower limbs and pelvis of P2. The radiographs of the lower limbs at 6/12 years **(A)**, and at 3 3/12 years **(B)**, and of the knees at 12 5/12 **(C)** and at 20 9/12 years **(D)**, and of the pelvis at 7 11/12 years **(E)**. The tubular bones are short and thick like those of P1. The evolution of the metaphyses and epiphyses is more abnormal than in P1 with more pronounced flaring and irregularity, and with corner fractures at the medial aspects of the distal femora and left proximal tibia, and, probably, of the medial femoral metaphyses as well. The metaphyseal and epiphyseal dappling resembles that of P1. In adulthood the left knee is almost normal, whereas the evaluation of the right knee, after the osteotomies of the proximal tibia, cannot be made. The iliac bones are small and rounded, sacrosciatic notches small, and acetabular roofs horizontal. The development of the irregular capital epiphyses is markedly delayed, the femoral necks are short and broad, and in varus position. The greater trochanters are located high. The lesser trochanters are spur-like.

cated [reviewed by Wolf, 1995]. Here we report on two individuals with SEMD due to a Gly154Arg substitution of type II collagen, a major structural protein of cartilage. These reports might give descriptive answers to the following three questions: 1) What are the similarities and dissimilarities of the clinical and radiographic phenotypes? 2) How do the clinical and radiographic manifestations evolve from birth to adulthood in this chondrodysplasia? 3) What are the similarities and dissimilarities of the phenotype of the present mutation as compared with the phenotypes due to other mutations in the *COL2A1* gene?

During the past few years a number of mutations in several genes have been found to cause various forms of chondrodysplasias. Mutations in cartilage collagen genes, such as *COL2A1, COL9, COL10A1,* and *COL11.* coding for structural proteins, cause chondrodysplasias in mice and men [reviewed by Prockop and Kivirikko, 1995]. Other genes involved in the chondrodysplasias code for membrane proteins or receptors. Mutations in such genes as in the *FGFR3* gene results in achondroplasia, hypochondroplasia, and thanatophoric dysplasia I and II; mutations in the *DTDST* gene cause diastrophic dysplasia, achondrogenesis IB, and atelosteogenesis II; and mutations in the *PTH-PTHrP* gene leads to metaphyseal chondrodysplasia, Jansen type [reviewed by Horton, 1995]. The heterozygous mutations in *FGFR3* gene result in a dominant-negative effect, whereas the conditions due to homozygosity of the mutations in *DTDST* gene are recessive traits.

The clinical phenotypes of P1 and P2 are markedly similar, as described above. The chondrodysplasia was observed at birth due to rhizomelia. Both had growth failure from birth to adulthood. Genua vara/valga deformities were similar, and the dissimilarities could be accounted for by the orthopedic procedures. The

Fig. 11. Upper limbs and hands of P2. Radiographs at 6/12 **(A)** and at 7 11/12 **(B)** years show short, thick, and poorly remodelled tubular bones, and there is a mild flaring changing to a severely irregular and dappled metaphyses of the distal ulnar and radius. The proximal humeral head is prominent. The tubular bones of the hand at 2 2/12 years of age **(C)** and 20 4/12 years **(D)** are normal. The carpal bones are only mildly dysplastic.

sacrolumbar lordosis of the patients was increased, but the thoracolumbar lordosis of P1 was mild. It is noteworthy that the propositi had no scoliosis or cleft palate. P1 had a pectus excavatum. The skeletal radiographic changes were similar with short, thick long bones, relatively mild abnormalities in the vertebrae, delayed development of epiphyses, and severe dysplasia of the hip joint. Peculiar findings were the marked metaphyseal and epiphyseal irregularities, or dappling, which developed in childhood and disappeared after closure of the growth plates. It has been suggested that alternating zones of osteosclerosis and osteopenia results in the dappling phenomenon [Anderson et al., 1982]. The corner fractures of the metaphyses were also a rarely observed radiographic change [Langer et al., 1990]. In adulthood, the radiographic phenotype of the knees was almost normal. The clinical and radiographic phenotype of the patients at birth and in infancy resembled that of SEDC (Table I). After the evolution of the metaphyseal dysplasia with dappling and corner fractures, the diagnosis changed into SMED, Strudwick type, or SMD, Sutcliffe type. In our original report on P1, we called his condition SED in spite of the marked involvement of the metaphyses [Vikkula et al., 1993]. The overall phenotype of the chondrodysplasia in P1 and P2 is relatively mild as compared with the descriptions on SMED, Strudwick type (Table I), but did not quite fit the descriptions of other SEMDs either, because of the dappling phenomenon. In 1982 Spranger and Maroteaux already questioned whether SEDC and SMED, Strudwick type, should not be separated [Spranger and Maroteaux, 1982]. There are increasing reports on mutations in the *COL2A1* gene in patients with various forms of chondrodysplasias; the clinical and radiographic spectrum of type II collagenopathies has become obvious [Spranger et al., 1994].

Type II collagen is an approximately 1,000 amino acid long triple helical molecule consisting of three identical α-chains [see Prockop and Kivirikko, 1995]. These α-chains consist of repeating tripeptide sequences of Gly-X-Y. The presence of glycine as every third amino acid in the tripeptide is essential because a large amino acid will not fit in the center of the triple helimutations that substitute bulkier amino acids for glycine residues can interrupt the folding of the triple helix and lead to the intracellular accumulation of unfolded procollagen. Some substitutions for obligate glycines can interrupt fibril assembly. Despite a considerable number of glycine substitutions already identified in the *COL2A1* gene, it is not possible to predict the biochemical consequences of these mutations. Glycine substitutions have been identified in various chondrodysplasias such as achondrogenesis, hypochondrogenesis, SEDC, and the Wagner syndrome [Prockop and Kivirikko, 1995; Vikkula et al., 1994]. The glycine substitution, Gly154Arg, in the present unrelated patients results in a moderately severe chondrodysplasia. On the other hand, a glycine to aspartate substitution at position α1-67 results in the type of the Wagner syndrome without cartilage manifestations [Körkkö et al., 1993]. The wide varia-

tion in the chondrodysplasia phenotypes in patients with glycine substitutions in the *COL2A1* gene is in agreement with the reports of glycine substitutions in the *COL1A1* and *COL1A2* genes in individuals with osteogenesis imperfecta and related phenotypes [Prockop and Kivirikko, 1995].

The spectrum of the type II collagenopathies ranges from lethal achondrogenesis II and hypochondrogenesis to SEDC, the Kniest dysplasia, the Stickler and Wagner syndromes, and familial precocious osteoarthrosis with mild chondrodysplasia (POA/SED). The ocular manifestations, i.e., myopia, vitreous syneresis, and retinal detachment, in type II collagenopathies have been explained by the presence of type II collagen in vitreous. Only P2 was myopic and had minor abnormalities in the retina and unilateral opacity in the lens, probably unrelated to her collagenopathy. Tiller et al. [1993] reported on posttranslationally modified α-1(II) collagen chains in two patients with SMED, Strudwick type, of which one had GGC-to-TGC transversion resulting a Gly709Cys substitution, but the phenotypes were not described in detail. However, they did mention the characteristic dappling of the metaphyses [Tiller et al., 1993]. It is not possible, based on the 2 observed mutations in the type II collagen molecule, Gly154Arg and Gly709Cys, to explain the resulting clinical and radiographic phenotypes. To date there are more than 50 mutations in the *COL2A1* gene that result in disease reported in the literature [reviewed by Prockop and Kivirikko, 1995]. It is of special note that in two instances a heterozygous deletion of the *COL2A1* gene resulted in Kniest dysplasia in a child, and the mosaicism of the parent for the same deletion caused the Stickler phenotype in the first family and a mild SEDC in the second family [Winterpacht et al., 1993, 1994].

There are reports on 4 unrelated families with the same Arg519Cys mutation of the *COL2A1* gene that results in familial POA/SED [Ala-Kokko et al., 1990; Pun et al., 1994; Williams et al., 1995]. The phenotype of the mutation has not been described in detail. The reports give an impression of generalized osteoarthritis with an onset in the second decade and progression to hip transplantation stage between the ages of 30 and 40 years, but affected relatives with Legg-Perthes disease during their first decade are also mentioned. In addition to the precocious joint disease, affected individuals present with platyspondyly and endplate irregularities of the vertebrae [Katzenstein et al., 1990; Pun et al., 1994]. Thus, it can be concluded that the possibly common mutation resulting in Arg519Cys substitution in type II collagen produces the POA/SED phenotype, whereas mutations in the *COL2A1* gene that decrease the cytoplasmic levels of type II collagen mRNA have been shown to produce the Stickler phenotype [reviewed by Vikkula et al., 1994; by Prockop and Kivirikko, 1995]. On the other hand, other factors than a specific mutation in collagen genes play a role in determining the resulting phenotype. As an example, the α1-Gly352Ser mutation in type I collagen produced a moderately severe osteogenesis imperfecta in two patients [Marini et al., 1993a; Bateman et al., 1993], a mild osteogenesis imperfecta in a third individual [Marini et al., 1993b], and a lethal osteogenesis imperfecta in a fourth [MacKay et al., 1993].

The type II collagenopathies constitute a continuum of skeletal and ocular diseases awaiting further genotype-phenotype studies.

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